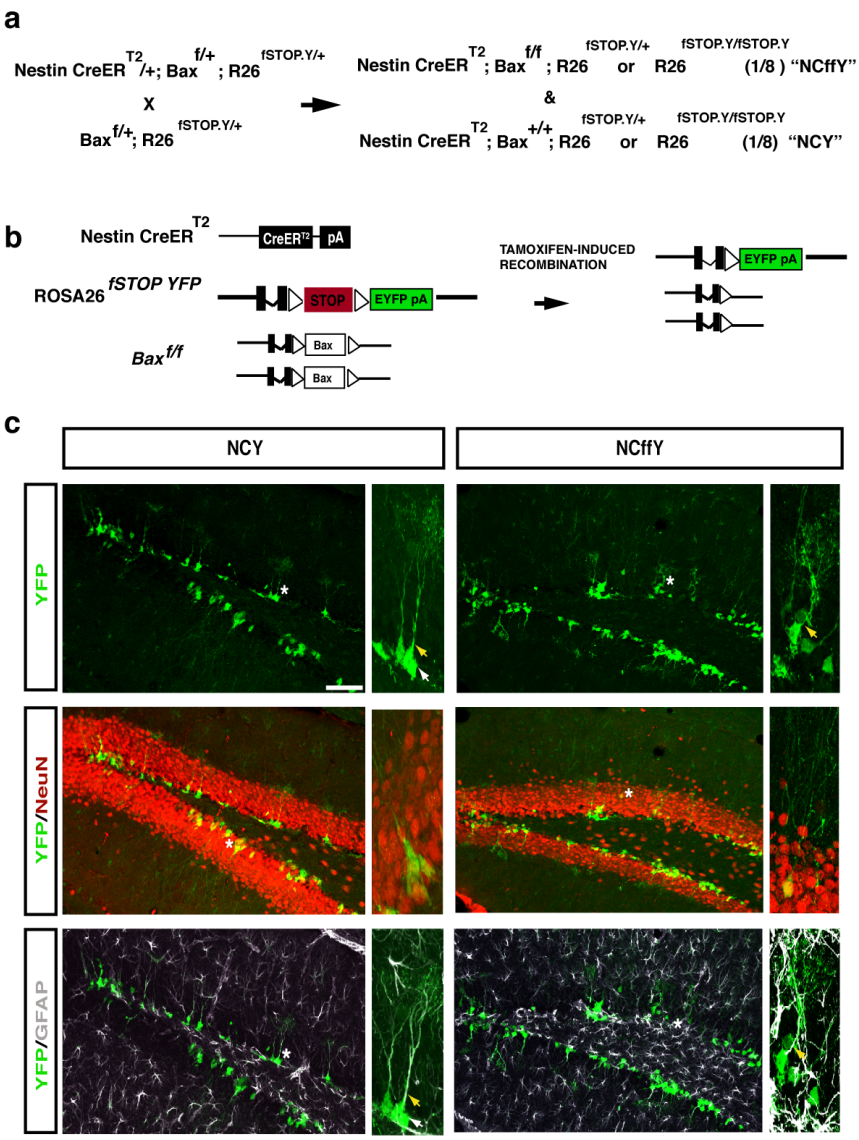
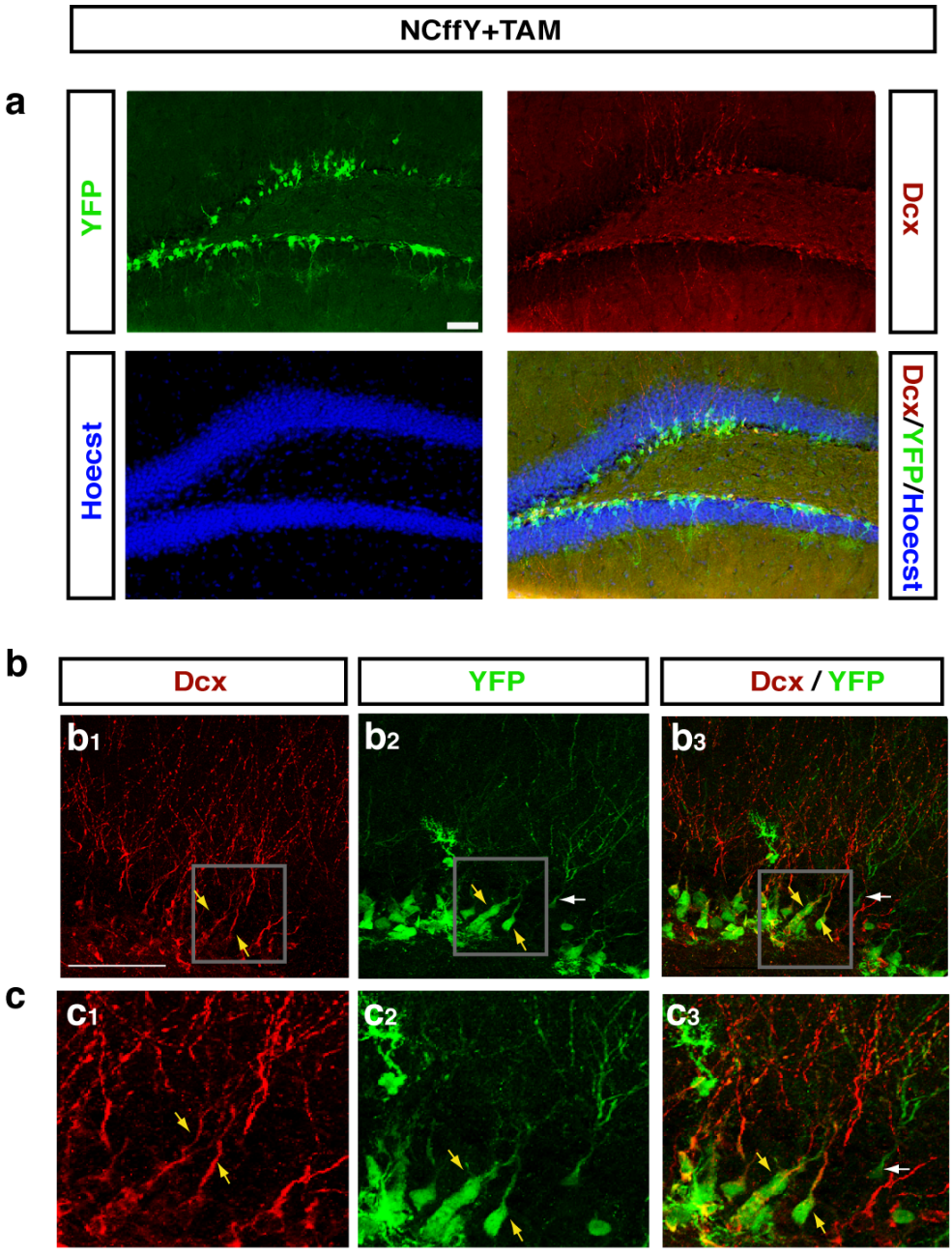
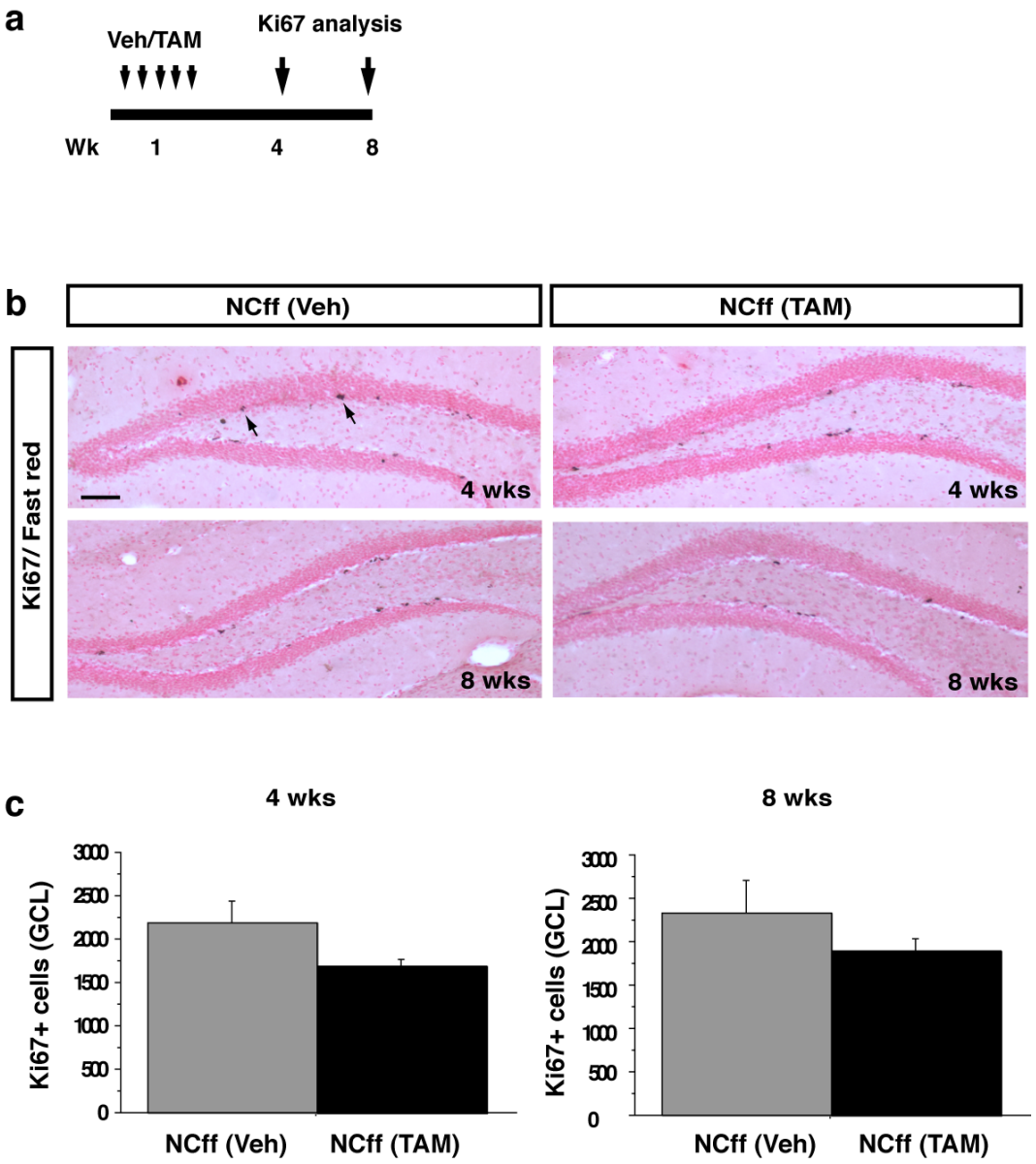
Supplementary Information for manuscript entitled "Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation" by Sahay et al., contains 21 Supplementary figures and accompanying figure legends.



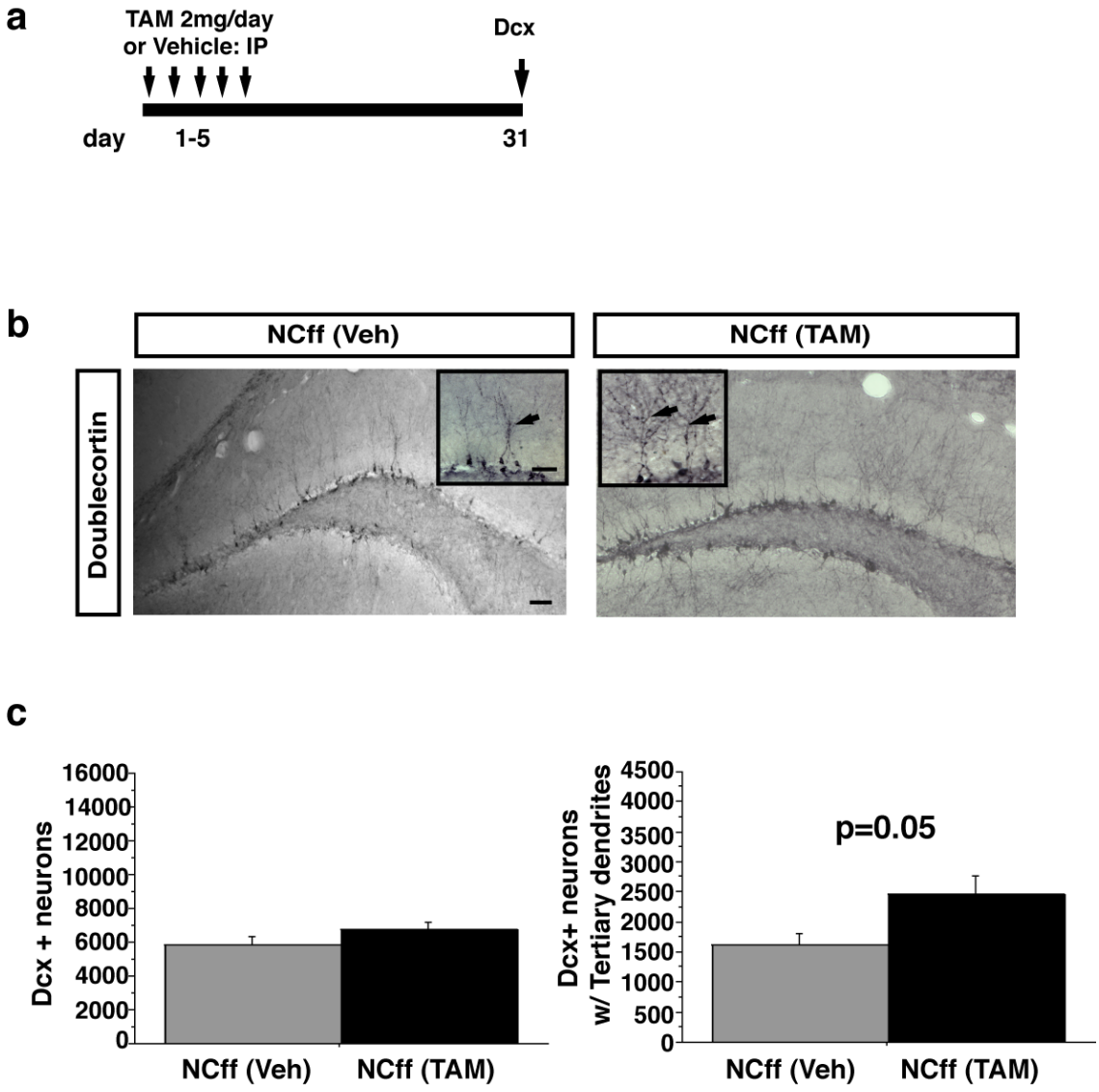
Supplementary Figure 1. Nestin CreER^{T2} dependent recombination is observed in type I neural stem cells and type II cells and their progeny in the DG of adult mice. **a**, Breeding strategy used to generate NCY and NCffY mice. Specifically, NCffY and NCY mice were generated from interbreeding *Nestin CreER*^{T2}; *Bax*$^{f/+}$; *ROSA26*$^{f STOP YFP/+}$ and *Bax*$^{f/+}$, *ROSA26*$^{f STOP YFP/+}$ mice. **b**, Schematic illustrating TAM induced recombination of *Bax*$^{f/f}$ and *ROSA26*$^{f STOP YFP/+}$ loci in NCffY mice. **c**, Confocal micrographs of coronal hippocampal sections of NCY and NCffY mice six weeks after TAM injections showing recombination in Type I (GFAP+,YFP+ with radial-glia like morphology, yellow arrow) neural stem cells and Type II cells (GFAP-, YFP+ with oval cell bodies, white arrow) in the subgranular zone and adult-born neurons (NeuN) in the DG. Scale bar 50μm.



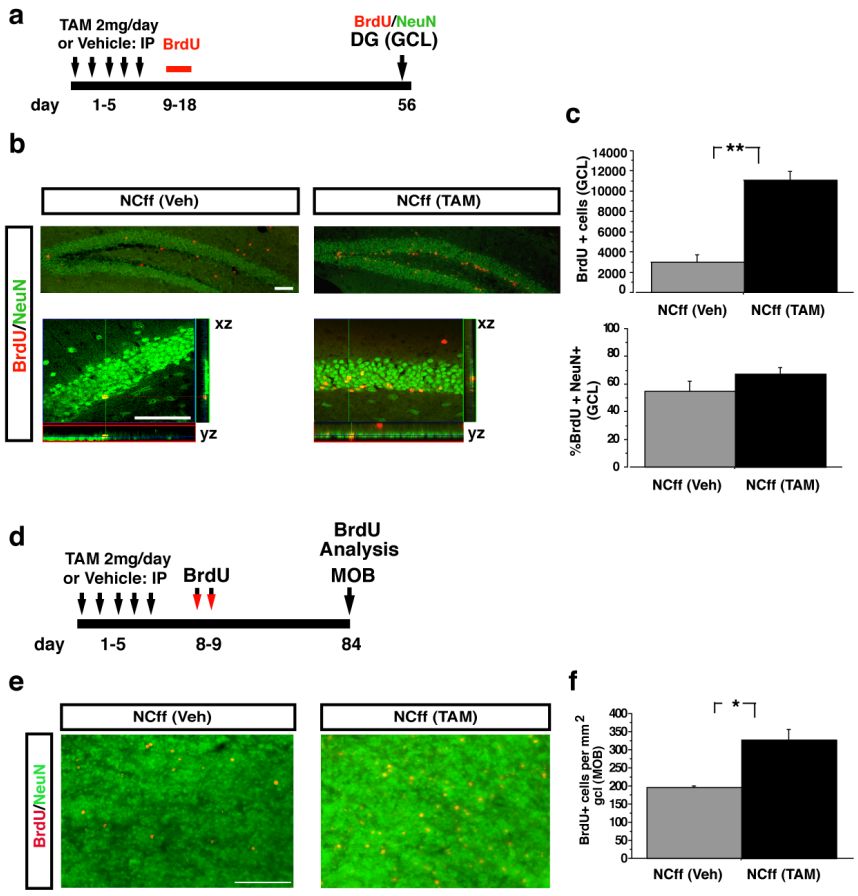
Supplementary Figure 2. a-b, Confocal micrographs of Dcx/YFP immunostained hippocampal sections of NCffY mice (6 weeks post TAM injection) showing overlap of Dcx and YFP. **c**, High magnification of area boxed in Panel b. Yellow arrows indicate Dcx neurons that also express YFP and therefore have presumably undergone recombination at the *Bax* conditional locus. White arrow indicates a YFP expressing mature adult-born neuron that is past the Dcx expressing stage. Scale bar 100μm.



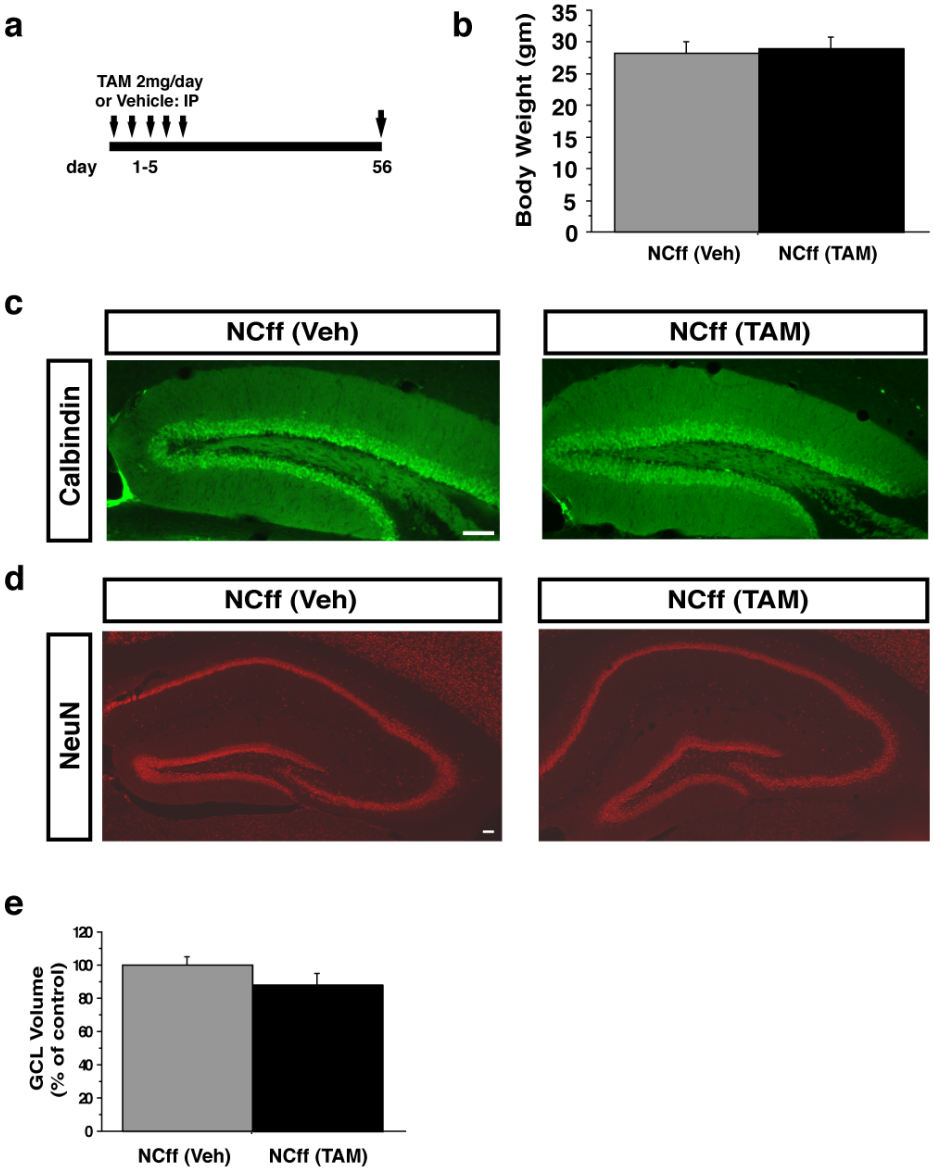
Supplementary Figure 3. *Bax* ablation in neural stem cells in the adult hippocampus does not impact progenitor proliferation. **a**, Experimental timeline. **b**, Representative Ki67 (arrows) immunostained coronal hippocampal sections of Veh and TAM treated NCff mice at 4 weeks and 8 weeks post Veh/TAM injection. **c**, Both groups show comparable levels of proliferation: ANOVA (treatment), 4 weeks, $F_{(1, 6)}=3$, $P=0.1$, 8 weeks, $F_{(1, 8)}=1$, $P=0.3$. n=4 (4 weeks) and 5 (8 weeks) mice per group. Results are mean ± SEM. Scale bar 100μm.



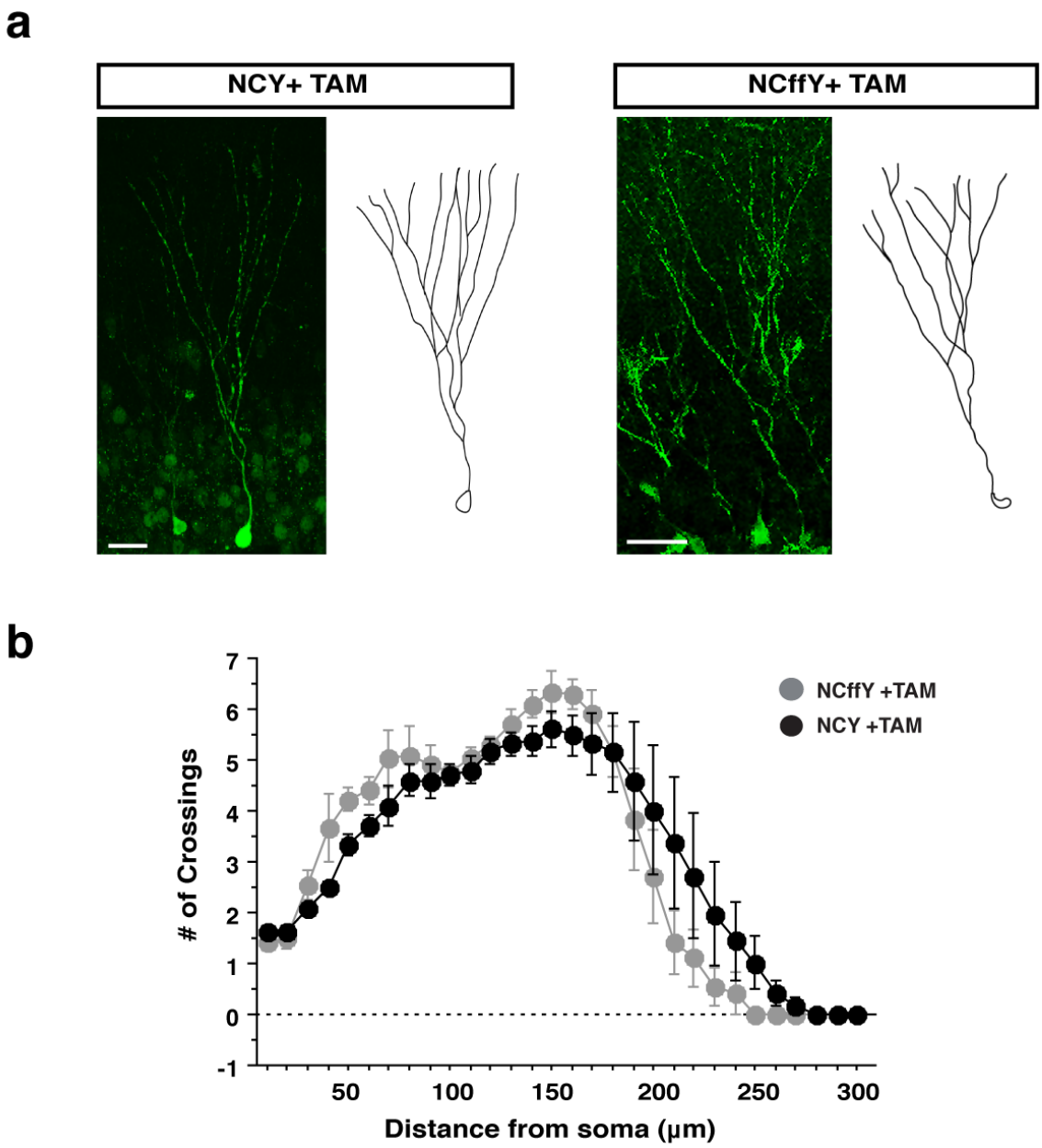
Supplementary Figure 4. *iBax*^{nestin} mice show an increase in the population of mature-Dcx expressing neurons 4 weeks following *Bax* ablation in stem cells in the adult brain. **a**, Experimental timeline. **b,** Representative Dcx immunostained coronal hippocampal sections of Veh and TAM treated NCff mice. Arrowheads in insets indicate Dcx neurons with at least tertiary dendrites. **c**, Quantification of Dcx population. Total Dcx+ neurons: 5866 ± 434 (NCff+Veh), 6787 ± 409 (NCff+TAM), ANOVA, $F_{(1, 6)}<1$. Dcx+ neurons with at least tertiary dendrites: 1620 ± 187 (NCff+Veh), 2469 ± 305 (NCff+TAM), ANOVA, $F_{(1, 6)}=5.6$, * $P=0.05$, n=4 mice per group. Results are mean ± SEM. Scale bar 100μm.



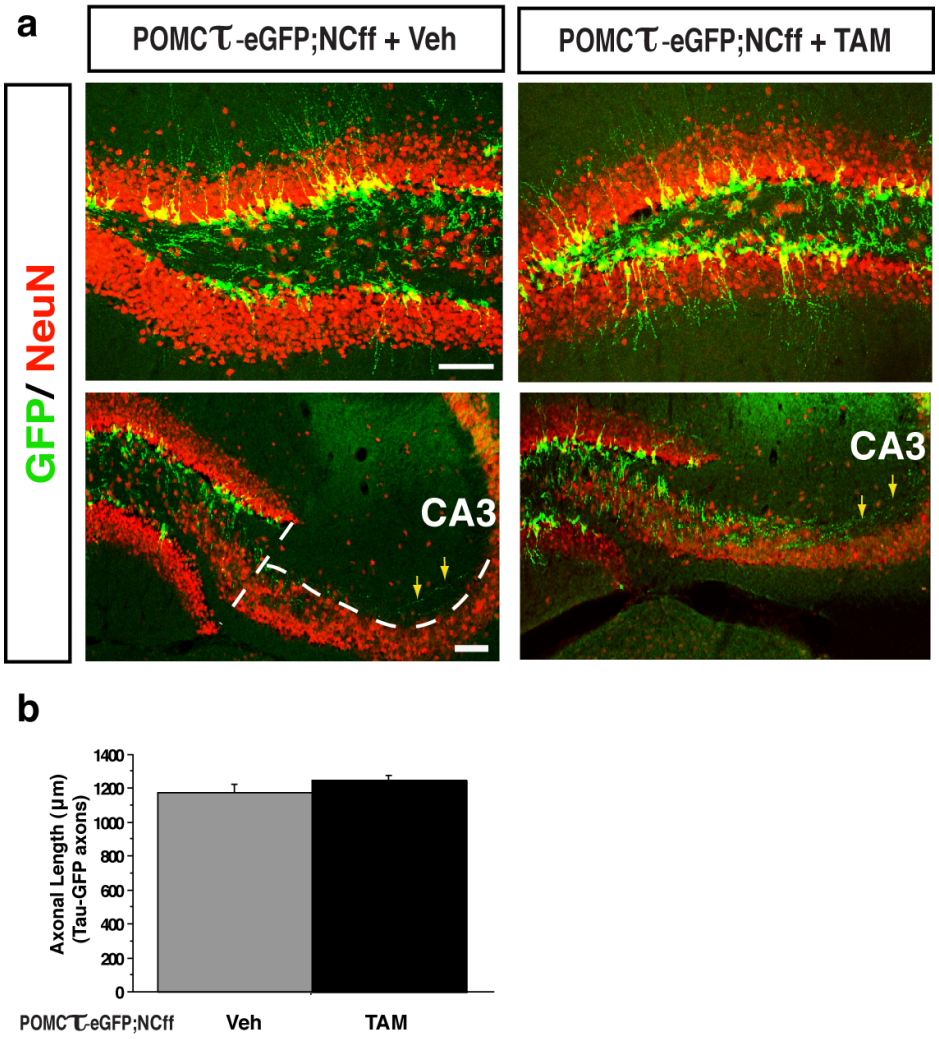
Supplementary Figure 5. *iBax*^{nestin} mice show enhanced long-term survival of adult-born neurons in the dentate gyrus and main olfactory bulb. **a**, Experimental design. **b**, Representative confocal micrographs of BrdU (red)/NeuN (green) immunostained coronal hippocampal sections of Veh and TAM treated NCff mice. **c**, Quantification of neuronal survival. Number of BrdU+ cells in the granule cell layer (GCL): 3004 ±733 (NCff+Veh), 11113 ± 874 (NCff+TAM), ** P=0.0004. Percentage of BrdU+NeuN+ neurons in GCL: 54.6 ± 7.3% (NCff+Veh), 67.2 ± 4.7% (NCff+TAM). **d**, Experimental design for quantification of subventricular zone derived adult-born neurons in main olfactory bulb. **e**, Representative BrdU/NeuN immunostained sagittal MOB sections of Veh and TAM treated NCff mice. **f**, NCff+TAM mice show a significant increase in the number of BrdU+ cells in MOB compared to NCff+Veh mice. BrdU+ cells per mm^2 of granule cell layer of MOB: 196.14 ± 4.5 (NCff+Veh), 328 ± 29 (NCff+TAM), ANOVA, $F_{(1, 4)}$=20.1, * P=0.01. n=3 mice per group. Results are mean ± SEM. Scale bars: (b) 100μm and 50μm, (e) 100μm.



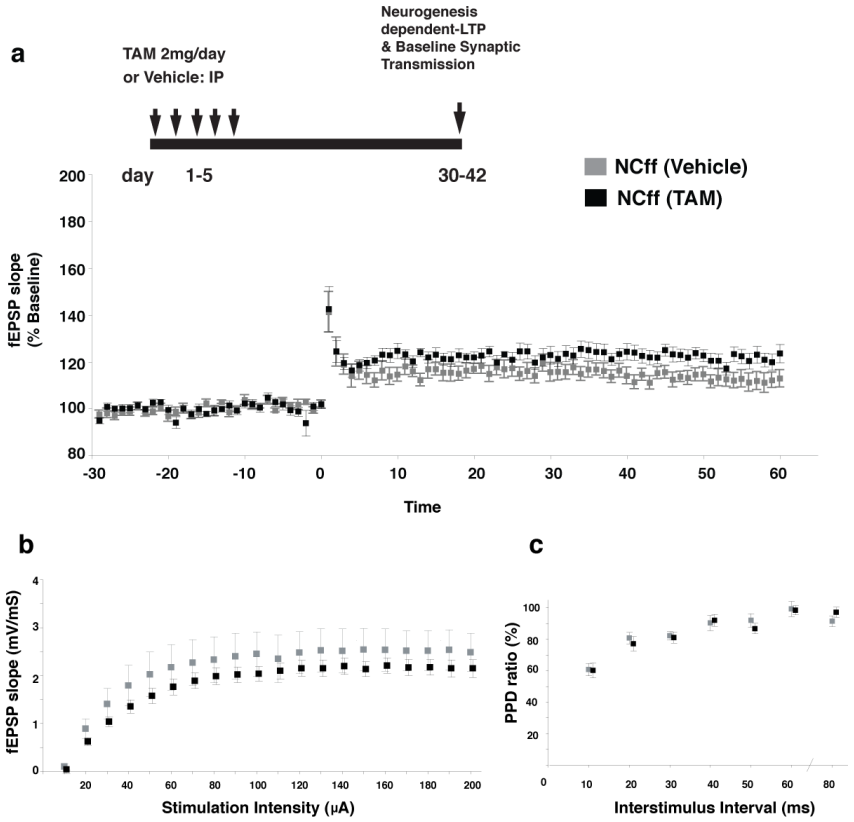
Supplementary Figure 6. *iBax*^{nestin} mice have normal body weight and DG and hippocampal architecture. **a**, Experimental timeline. **b**, NCff+Veh (n=9) and NCff+TAM (n=11) mice have comparable body weights. **c-d**, Expression of Calbindin and NeuN is unaffected by *Bax* ablation in adult stem cells. **e**, The dentate gyrus granule cell layer of NCff+Veh (n=3) and NCff+TAM (n=4) mice have comparable volumes (8-12 weeks post Veh/TAM injections). Results are mean ± SEM. Scale bar 100μm.



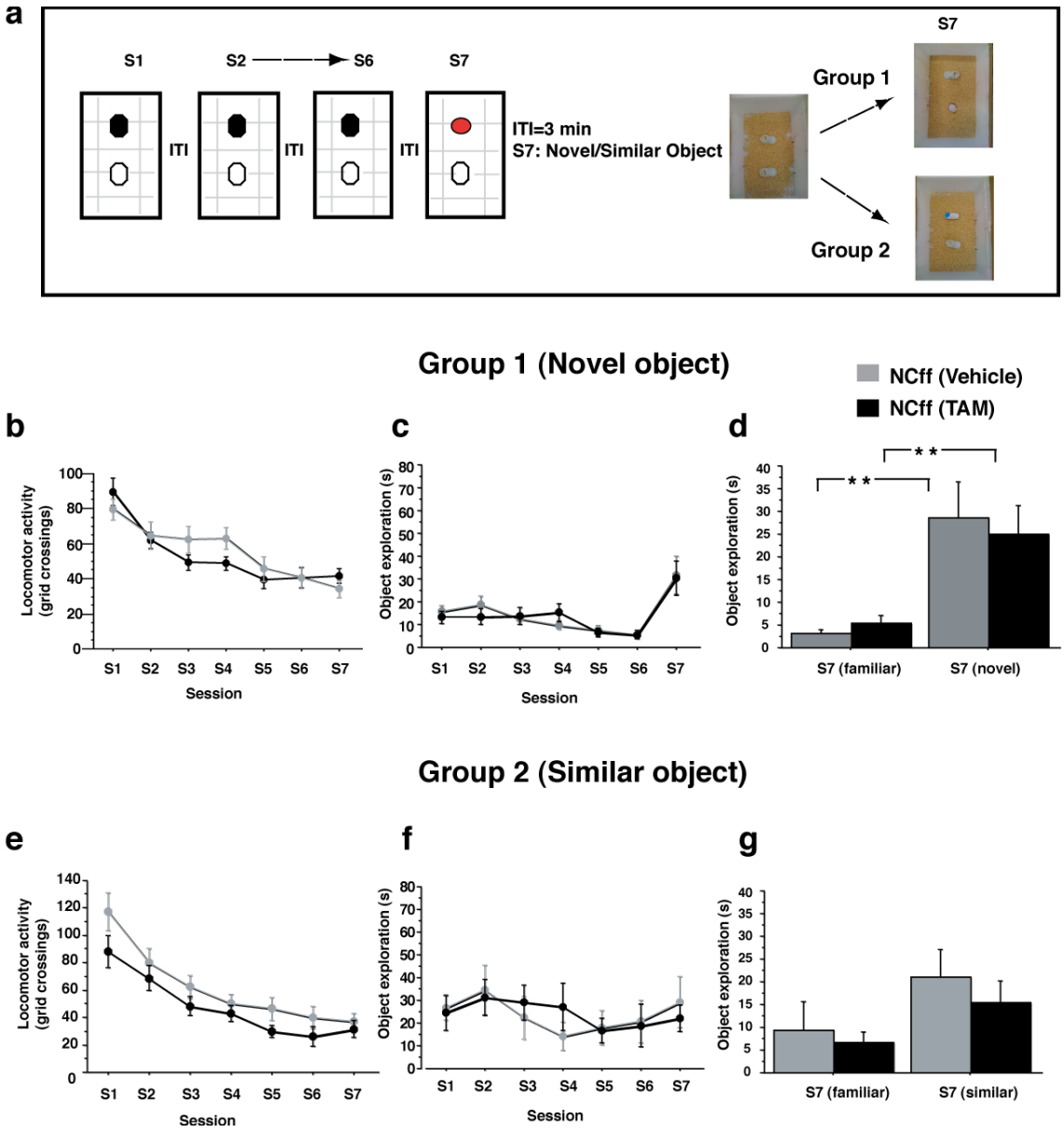
Supplementary Figure 7. Sholl analysis of 6 weeks old adult-born neurons in NCY and NCffY mice. **a**, Sample projection of Z series confocal images of a YFP expressing adult-born neuron in the DG of NCY and NCffY mice. On right of each neuron is the corresponding 2D projection trace from the 3D confocal reconstruction of dendrites. **b**, Adult-born neurons in NCY and NCffY mice show similar dendritic complexity as assessed by Sholl analysis. Repeated measures ANOVA (genotype) $F_{(1, 5)}<1$, $P=0.9$. n=3 and 4 mice per group. Results are mean ± SEM. Scale bar 100µm.



Supplementary Figure 8. Normal axonal targeting of mossy fibers of adult-born neurons following *Bax* ablation in neural stem cells in the adult brain. **a**, Representative confocal micrographs of hippocampal sections of NCff; POMC-τ-eGFP mice six weeks following Veh/TAM treatment showing GFP labeled adult-born neurons and their axons (yellow arrows). Dashed line in left lower panel indicates measurement procedure for mossy fiber length. **b**, Length of mossy fibers of young adult-born neurons of Veh and TAM treated mice: 1173 ± 54 µm (Veh), 1246 ± 33 µm (TAM), ANOVA, $F_{(1, 5)}$=1.1, *P*=0.34. n=3 and 4 mice per group. Results are mean ± SEM. Scale bar 100µm.

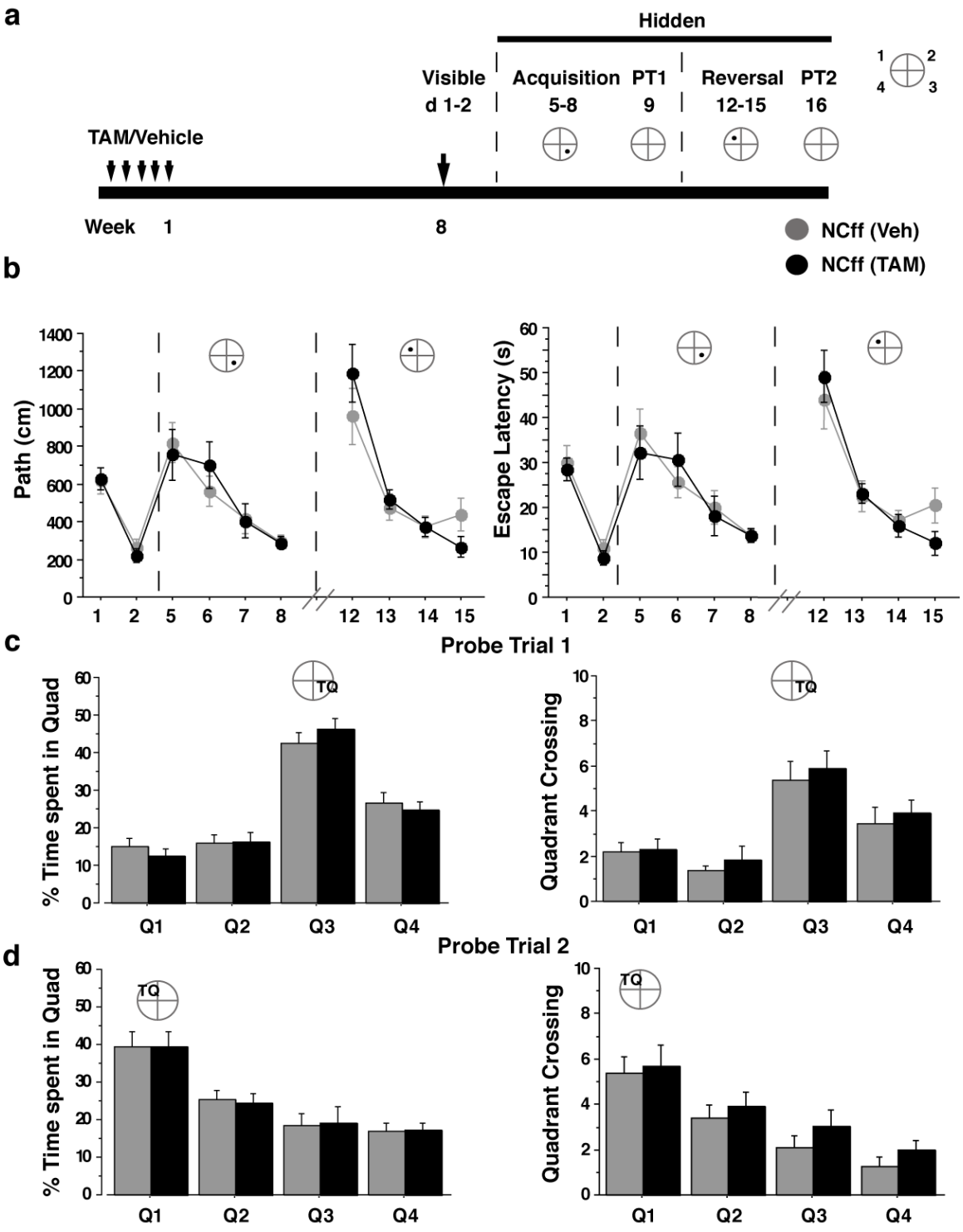


Supplementary Figure 9. *iBax*^{nestin} mice show increased neurogenesis dependent LTP and normal baseline synaptic transmission at MPP-DG synapses at 4-6 weeks following Veh/TAM treatment. **a**, NCff+TAM mice show enhanced medial perforant path-dentate gyrus LTP compared to NCff+Veh mice. Repeated measures ANOVA over 50 minutes of recording revealed a significant effect of treatment, $F_{(1, 17)}$=4.7, *P*=0.04. n=10 slices, 5 mice (Veh), n=9 slices, 6 mice (TAM). **b**, Input-output relationship was not different between NCff+Veh and NCff+TAM mice, ANOVA $F_{(1, 17)}$<1. **c**, No differences were observed for paired-pulse depression ratios obtained from NCff+Veh and NCff+TAM mice for any interstimulus interval tested, p>0.05, unpaired 2-tailed Student's t-tests. n=10 slices from 5 mice (Veh), n=9 slices from 6 mice (TAM). Results are mean ± SEM.



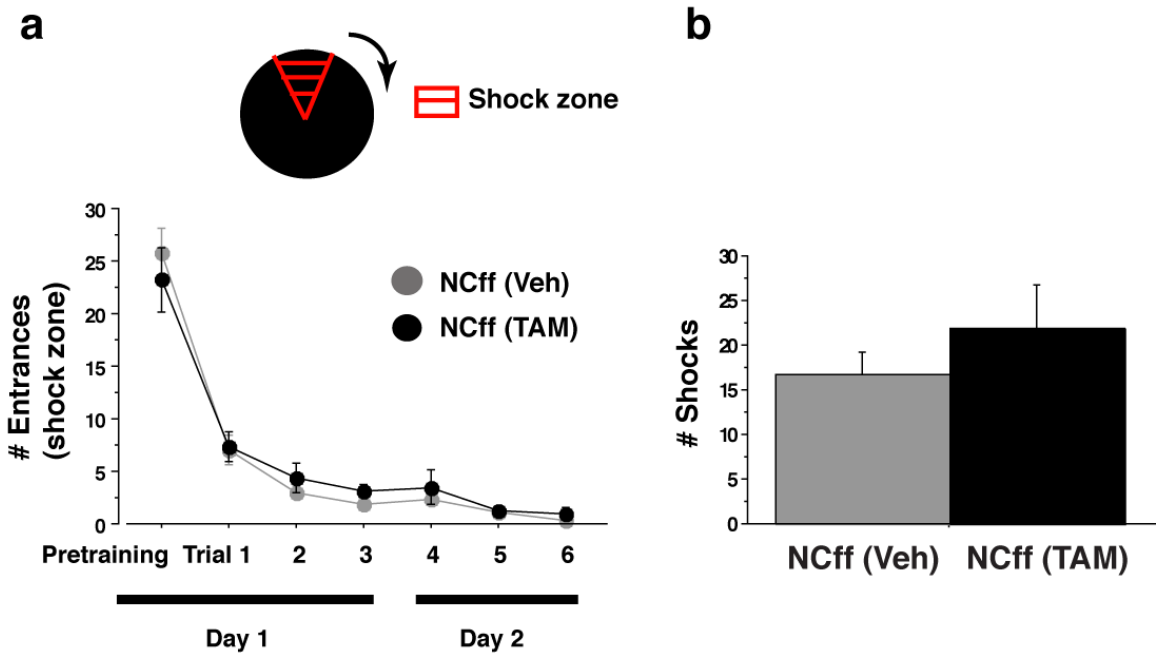
Supplementary Figure 10. Increasing adult hippocampal neurogenesis does not impact object recognition behavior. **a**, Schematic of experimental design to test novel and similar object recognition. **b-d**, Both NCff+Veh and NCff+TAM showed comparable levels of novel object recognition. **b**, NCff+Veh and NCff+TAM mice showed similar habituation of locomotor activity [(Two-way repeated measures ANOVA, (treatment) $F_{(1, 22)}<1$, (session) $F_{(6, 132)}=40$, $P<0.0001$, (treatment X session) $F_{(6, 132)}=3.3$, $P=0.004$)]. **c**, Both groups of mice exhibited similar levels of novel object-exploration behavior [(Two-way

repeated measures ANOVA, (treatment) $F_{(1, 22)}<1$, (session) $F_{(6, 132)}=15.61$, $P<0.0001$, (treatment X session) $F_{(6, 132)}<1$)]. **d**, Both groups showed similar levels of recognition of novel object that were significantly greater than exploration of familiar object [(Two-way ANOVA (exploration) $F_{(1, 22)}=18.9$, $P<0.0001$, (treatment) $F_{(1, 22)}<1$, (treatment X exploration) $F_{(1, 22)}<1$)]. Exploration time: NCff+Veh (familiar, 3.13 ± 0.76 sec, novel, 28.51 ± 8 sec), NCff+TAM (familiar, 5.47 ± 1.6 sec, novel, 24.95 ± 6.2 sec). Exploration of novel object compared to familiar object in session 7: NCff +Veh (** $P=0.0047$), NCff +TAM (** $P=0.006$)]. n=12 mice per gp. **e-g**, A separate cohort of mice was tested using a similar object that evoked lower levels of exploration than a novel object. NCff+Veh (n=8) and NCff+TAM (n=11) mice showed comparable locomotor activity and habituation of this behavior [(Two-way repeated measures ANOVA, (treatment) $F_{(1, 17)}=2.21$, $P=0.15$, (session) $F_{(6, 102)}=39.8$, $P<0.0001$, (treatment X session) $F_{(6, 102)}<1$)] as well as exploration of the similar object [(Two-way repeated measures ANOVA, (treatment) $F_{(1, 17)}<1$, (session) $F_{(6, 102)}=2.2$, $P=0.04$, (treatment X session) $F_{(6, 102)}<1$)] and similar levels of object exploration in session 7 [(Two-way ANOVA, (treatment) $F_{(1, 17)}<1$, (exploration) $F_{(1, 17)}=4.5$, $P=0.04$, (treatment X session) $F_{(1, 17)}<1$)]. Results are mean ± SEM.

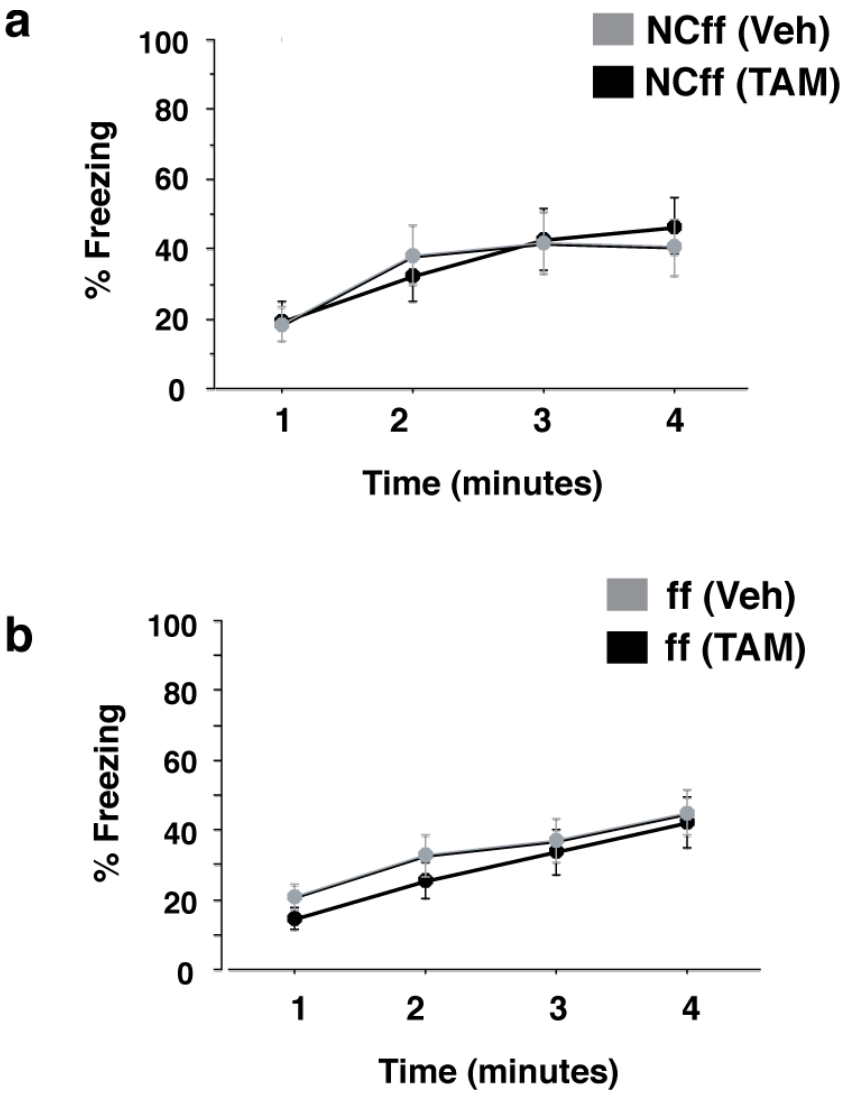


Supplementary Figure 11. Increasing adult hippocampal neurogenesis does not impact spatial and reversal learning and memory. **a**, Experimental design and timeline. **b**, Acquisition curves plotting path length and escape latencies during visible, acquisition and

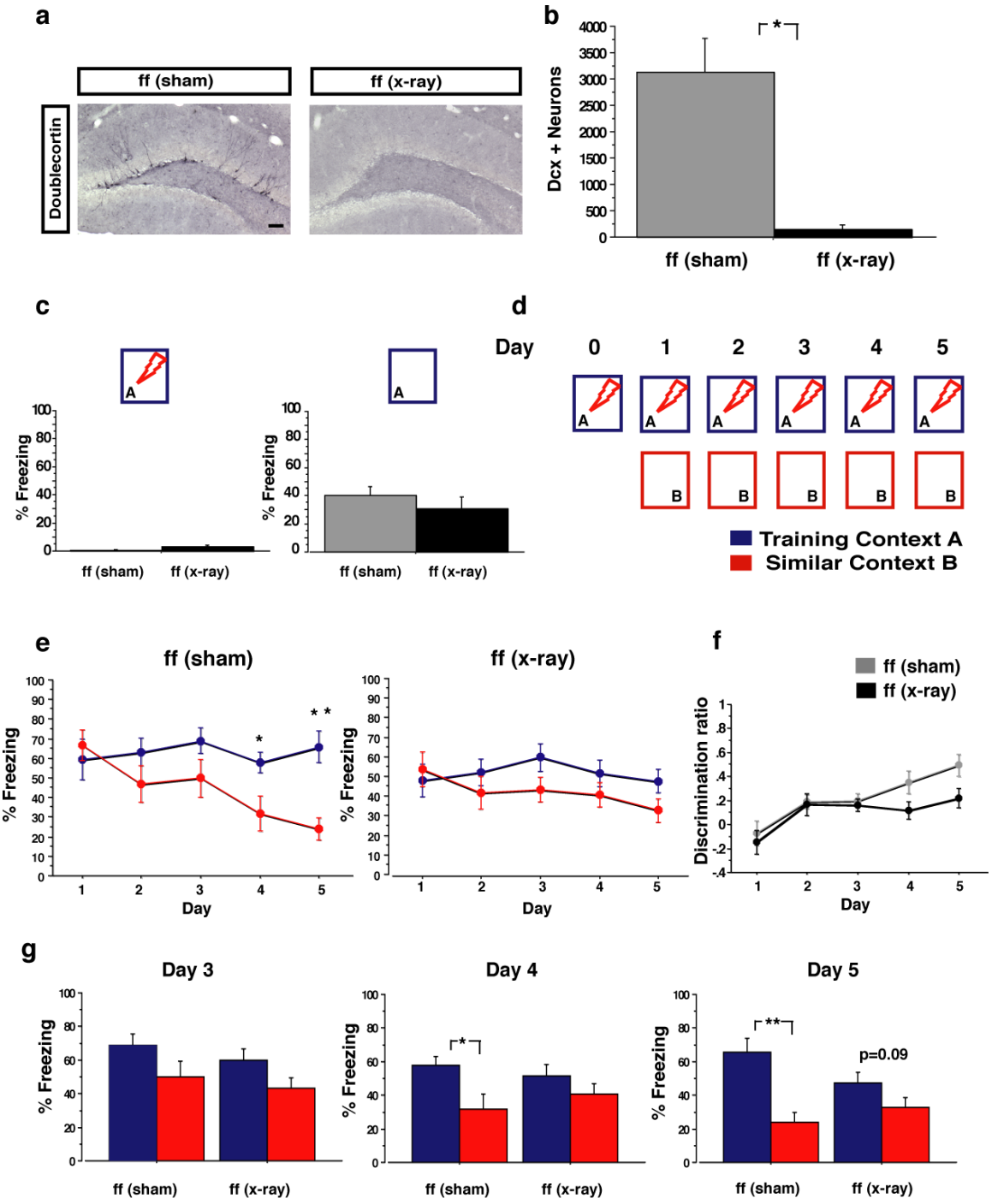
reversal phase of experiment. Both groups learned to swim to the marked platform equally well [(Visible d1-d2, Two-way repeated measures ANOVA for path: (treatment) $F_{(1, 19)}<1$, $P=0.7$, (treatment X day) $F_{(1, 19)}<1$, $P=0.62$, (day) $F_{(1, 19)}= 50$, $P<0.0001$. Two-way repeated measures ANOVA for Escape latency: (treatment) $F_{(1, 19)}<1$, $P=0.5$, (treatment X day) $F_{(1, 19)}<1$, $P=0.87$, (day) $F_{(1, 19)}= 54$, $P<0.0001$]. During the acquisition phase d5-d8, both groups of mice acquired the location of the hidden platform in quadrant 3 at the same rate. [Two-way repeated measures ANOVA for path: (treatment) $F_{(1, 19)}<1$, $P=0.8$, (treatment X day) $F_{(3, 57)}<1$, $P=0.72$, (day) $F_{(3, 57)}= 11.7$, $P<0.0001$. Two-way repeated measures ANOVA for escape latency: (treatment) $F_{(1, 19)}<1$, $P=0.9$, (treatment X day) $F_{(3, 57)}<1$, $P=0.72$, (day) $F_{(3, 57)}= 9.2$, $P<0.0001$]. In the transfer/reversal phase d12-d15, both groups acquired the new location of the hidden platform in quadrant 1 at comparable rates. [Two-way repeated measures ANOVA for path: (treatment) $F_{(1, 19)}<1$, $P=0.75$, (treatment X day) $F_{(3, 57)}=1.7$, $P=0.17$, (day) $F_{(3, 57)}= 29.9$, $P<0.0001$. Two-way repeated measures ANOVA for escape latency: (treatment) $F_{(1, 19)}<1$, $P=0.8$, (treatment X day) $F_{(3, 57)}=1.2$, $P=0.3$, (day) $F_{(3, 57)}= 30.5$, $P<0.0001$]. **c**, Spatial memory of the location of the hidden platform was similar for both groups as reflected in comparable amount of time spent searching for the platform in the target quadrant (TQ)(NCff+Veh: 42.5 ± 2.6 % of time, NCff+TAM: 46.38 ± 2.62 % of time) and TQ crossings (NCff+Veh: 5.3 ± 0.845, NCff+TAM: 5.9 ± 0.752) in the probe trial (PT1) on day 9. Both groups showed similar thigmotaxis (ANOVA, $F_{(1, 19)} =0.004$, $P=0.9$) and floating (ANOVA, $F_{(1, 19)} =0.07$, $P=0.8$) behaviors. **d**, In Probe trial 2 (PT2) performed following the reversal phase on d16, spatial memory of the location of the hidden platform was similar for both groups as reflected in comparable amount of time spent searching for the platform in the target quadrant (TQ)(NCff+Veh: 39.43 ± 4 % of time, NCff+TAM: 39.2 ± 4.2 % of time) and TQ crossings (NCff+Veh: 5.3 ± 0.7, NCff+TAM: 5.7 ± 0.9). Both groups showed similar thigmotaxis (ANOVA, $F_{(1, 19)} =2.8$, $P=0.1$) and floating (ANOVA, $F_{(1, 19)} =0.7$, $P=0.4$) behaviors. n=11 (Veh) and 10 (TAM) mice. Results are mean ± SEM.



Supplementary Figure 12. Increasing adult hippocampal neurogenesis does not affect spatial learning in the active place avoidance task. Both groups of mice received comparable number of shocks and learned to avoid the nonrotating shock zone at similar rates [Two-way repeated measures ANOVA for number of entrances: (treatment) $F_{(1, 12)}$=0.7, *P*=0.4, (treatment X trial) $F_{(5, 60)}$=0.2, *P*=0.95, (trial) $F_{(5, 60)}$= 17, *P*<0.0001]. n=6 (Veh) and 8 (TAM) mice. Results are mean ± SEM.



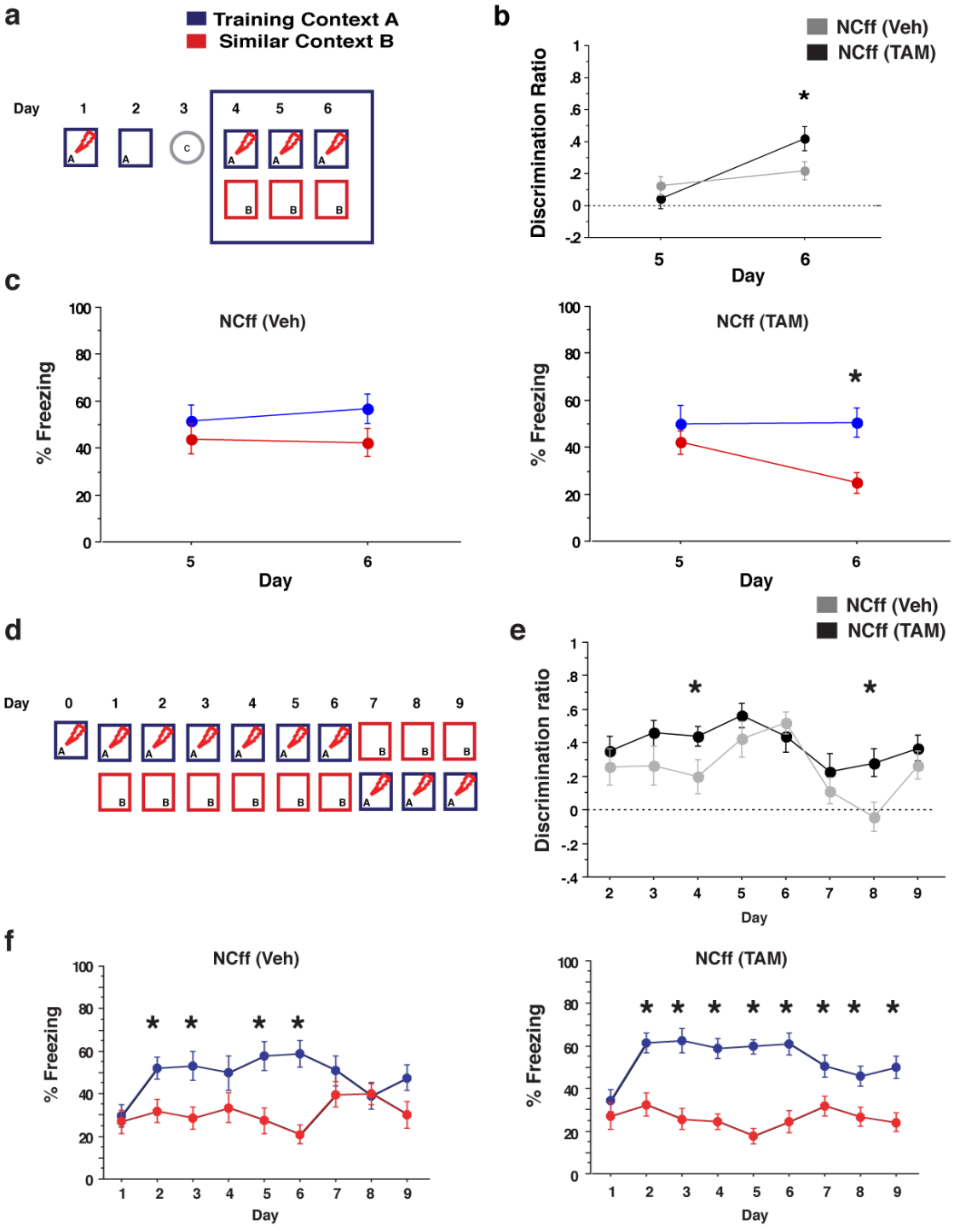
Supplementary Figure 13. Kinetics of freezing in training context of TAM and Vehicle treated NCff and ff mice. **a**, NCff+Veh and NCff+TAM mice showed identical kinetics of freezing in context A, 24 hours following conditioning with a single 2 second 0.75mA foot shock in A [(Two-way repeated measures ANOVA (treatment X time) $F_{(3, 78)}<1$, (treatment) $F_{(1, 26)}<1$, (time) $F_{(3, 78)}=17$, $P<0.0001$)], n=14 mice per group. **b,** TAM treatment on its own does not affect contextual fear conditioning in control "ff" mice. Both ff+Veh (n=15) and ff+TAM (n=16) mice showed comparable kinetics of freezing behavior [(Two-way repeated measures ANOVA (treatment X time) $F_{(1, 29)}<1$, (treatment) $F_{(3, 87)}<1$, (time) $F_{(3, 87)}=19.6$, $P<0.0001$)]. Results are mean ± SEM.



Supplementary Figure 14. Adult hippocampal neurogenesis is necessary for contextual fear discrimination learning. **a-b**, Low-dose hippocampal x-irradiation of "*Bax*^{ff}" mice blocks production of adult-born neurons. Total Dcx+ neurons: 3120 ± 659 (sham), 144 ± 75 (x-ray)[ANOVA, $F_{(1,4)}$=20.07, * P=0.011]. n=3 mice per group. **c**, Hippocampal x-irradiated mice show normal contextual fear conditioning using a single foot shock-context

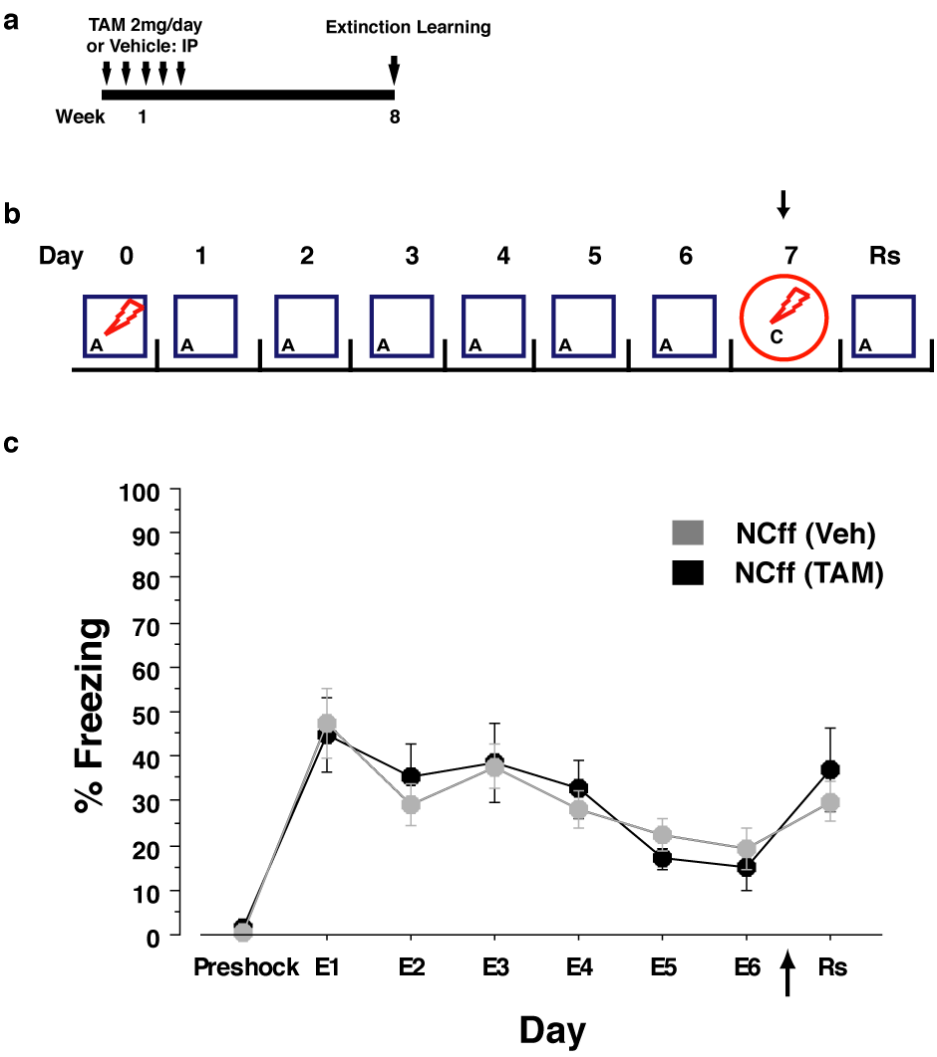
pairing protocol. [Freezing behavior in training context, ANOVA, $F_{(1, 15)}<1$, Average Freezing: 39.87 ± 6.7% (sham, n=8), 30.76 ± 8.24% (x-ray, n=9)]. **d**, Mice were tested in a contextual fear discrimination learning paradigm following contextual fear conditioning. **e,** Freezing behavior of sham (n=7) and hippocampal x-irradiated (n=9) mice in both contexts over duration of experiment. There is no difference in freezing behavior of both groups on Day 0 (not plotted here): ff (sham): 42.1 ± 10.2%, ff (x-ray): 39.3± 10.2%. On day 1, both groups showed comparable levels of generalization between the two contexts (Two-way ANOVA of Context and Treatment, (context) $F_{(1, 14)}<1$, (treatment) $F_{(1, 14)}<1$, (context X treatment) $F_{(1, 14)}<1$)]. Analysis of freezing behavior in both contexts over days revealed that ff (sham) mice were able to distinguish between contexts A and B by day 4 of contextual fear discrimination fear learning. In contrast, ff (x-ray) mice showed a trend towards lower freezing in context B relative to context A only on day 5 of contextual fear discrimination fear learning [(Two-way repeated measures ANOVA of Context and Day followed by Fisher's PLSD *post hoc* tests, ff (sham): (context) $F_{(1, 12)}=5.2$, $P=0.04$, (day) $F_{(4, 48)}=3.9$, $P=0.008$, (context X day) $F_{(4, 48)}=4.4$, $P=0.004$ and ff (x-ray): (context) $F_{(1, 16)}=1.25$, $P=0.3$, (day) $F_{(4, 64)}=2$, $P=0.1$, (context X day) $F_{(4, 64)}=2$, $P=0.1$)]. **f**, Comparison of discrimination ratios of ff (sham) relative to ff (x-ray) mice. Two-way repeated measures ANOVA of Treatment [(treatment) $F_{(1, 14)}= 3.34$, $P=0.1$, (day) $F_{(4, 56)}= 11$, $P<0.0001$, (treatment X day $F_{(4, 56)}=1.2$, $P=0.3$)] **g**, By day 5, ff (sham) mice displayed robust discrimination between the two contexts in contrast to ff (x-ray) mice, [(Two-way ANOVA of Context and Treatment, (context X treatment) $F_{(1, 14)}= 4.34$, $P=0.04$)].

Comparison of freezing behavior of both groups in similar context B over days (not shown above) showed a faster decrease in freezing levels of sham treated mice relative to x-irradiated mice [(Two-way repeated measures ANOVA of Treatment over days, (day) $F_{(4, 56)=}14.5$, $P=<0.0001$, (treatment X day) $F_{(4, 56)=}2.4$, $P=0.05$)]. In contrast, freezing behavior of hippocampal x-irradiated and sham treated mice in the training context A was not different [(repeated measures ANOVA of Treatment over days, (day) $F_{(4, 56)=}1$, $P=0.38$, (treatment X day), $F_{(4, 56)}< 1$, $P=0.9$)]. *$P<0.05$, **$P<0.01$. Results are mean ± SEM.

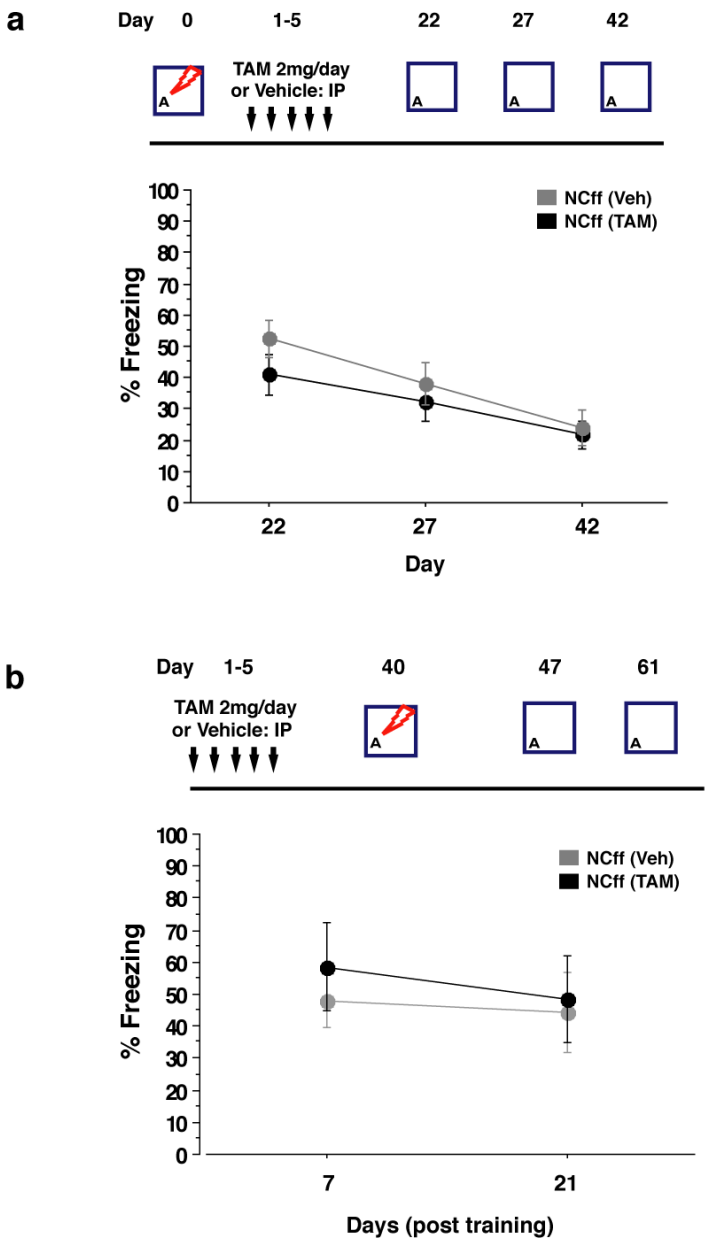


Supplementary Figure 15. Mice with more adult-born neurons exhibit better contextual fear discrimination learning than controls. **a**, Experimental design. Mice were subjected to a contextual fear discrimination learning paradigm (d4-d6) 24 hours following completion of contextual fear conditioning (d1-d3). **b**, Analysis of discrimination ratios.

Two-way repeated measures ANOVA of Treatment over days revealed a significant interaction between treatment and day and a significant main effect of day [(treatment) $F_{(1, 26)}<1$, $P=0.38$, (day) $F_{(1, 26)}=17.75$, $P=0.0003$, (treatment X day) $F_{(1, 26)}= 6.1$, $P=0.02$)]. Inspection of discrimination ratios on individual days using Fisher's PLSD *post hoc* tests revealed higher levels of discrimination of NCff+TAM relative to NCff+Veh mice on day 6 (*P =0.03). **c**, Freezing behavior of NCff (Veh and TAM) mice in contexts A and B. Three-way repeated measures ANOVA of Context and Treatment over days revealed a significant main effect of context and significant interactions between day and treatment as well as between day and context [(context) $F_{(1, 52)}=5.85$, $P=0.01$, (day) $F_{(1, 52)}=1.9$, $P=0.15$, (day X treatment) $F_{(1, 52)}= 4.5$, $P=0.03$, (context X day) $F_{(1, 52)}= 6.6$, $P=0.01$]. Fisher's PLSD *post hoc* tests showed that NCff+TAM mice, unlike controls, distinguished between contexts A and B on day 6. Furthermore, NCff+TAM mice showed significantly lower levels of freezing in context B relative to controls on day 6 (ANOVA, $F_{(1, 26)}=5.5$, $P=0.02$) (not shown). NCff (Veh/TAM)=14 per group**.** **d**, Experimental design. Note that order of context presentation is reversed on day 7 of testing. **e**, Analysis of discrimination ratios shows that NCff+TAM mice exhibit higher levels of discrimination than NCff+Veh mice and that NCff+Veh mice gradually acquired comparable levels of discrimination by day 6 and day 9. Two-way repeated measures ANOVA of Treatment over days three to nine revealed a trend towards a main effect of treatment [(treatment) $F_{(1, 22)}=3.6$, $P=0.06$], a significant main effect of day [(day) $F_{(6, 132)}=8.3$, p=<0.0001] and a significant interaction between treatment and day [(treatment X day) $F_{(6, 132)}=2.3$, $P=0.04$)]. Comparison of discrimination ratios on each day was performed using Fisher's PLSD *post hoc* tests. **f**, Freezing behavior of NCff (Veh and TAM) mice over duration of experiment. Two-way repeated measures ANOVA of Context over duration of testing revealed a significant interaction between day and context in each group [(NCff+Veh, (context X day) $F_{(8, 160)}=4.9$, $P<0.0001$)] and [(NCff+TAM, (context X day) $F_{(8, 192)}=4$, $P=0.0002$)]. Analysis of freezing behavior on each day by Fisher's PLSD *post hoc* tests revealed that NCff+TAM mice, unlike controls, exhibited significantly lower levels of freezing in context B relative to context A on days 2-9 and even when the order of context presentation was reversed. *$P<0.05$. Results are mean ± SEM.

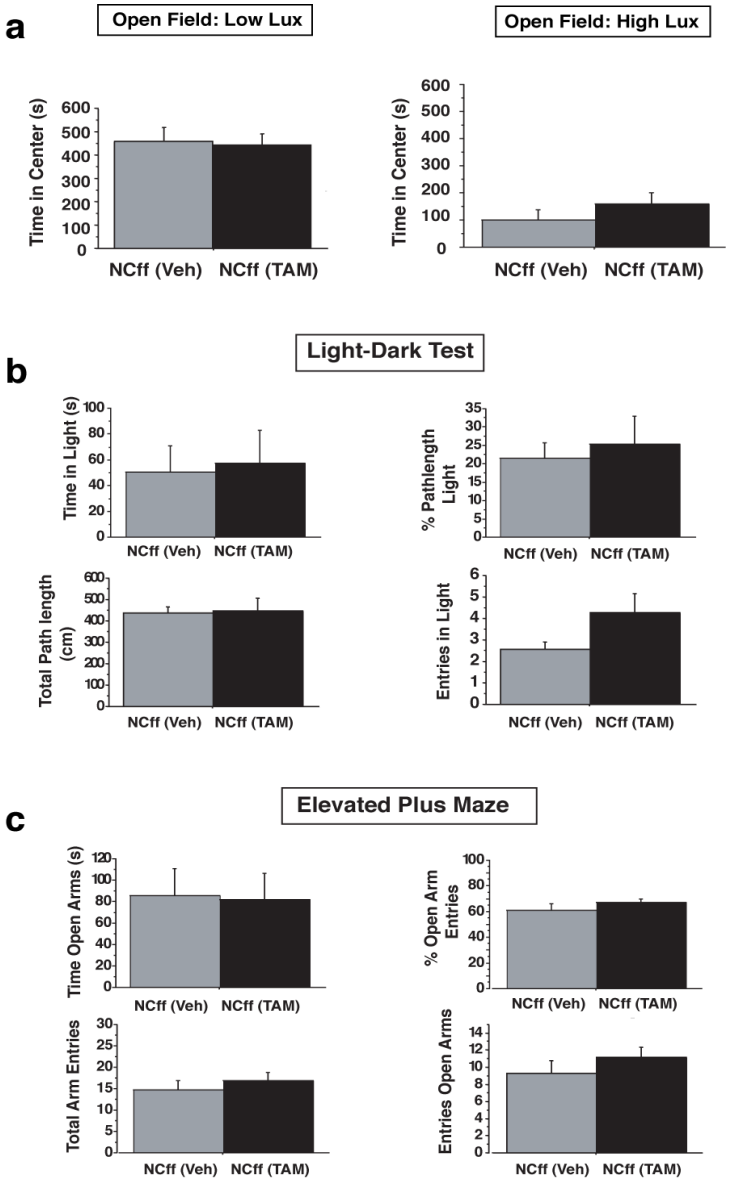


Supplementary Figure 16. *iBax*^{nestin} mice show normal extinction of learned contextual fear. **a**, Experimental timeline. **b**, Design of extinction learning paradigm. NCff+Veh (n=11) and NCff +TAM (n=9) mice were given a single foot shock (2 seconds, 0.75mA) 185 seconds following placement in context A and then subjected to a single extinction trial (3 minute re-exposure to context A without presentation of foot shock) daily for six consecutive days. Reinstatement of freezing behavior (Rs) was assessed 24 hours following presentation of a single foot shock in a novel context C on day 7. **c**, Both NCff+Veh and NCff+TAM mice showed similar freezing behavior during extinction learning and reinstatement [(Two-way repeated measures ANOVA, (treatment) $F_{(1, 18)}$=0.01, P=0.8, (day) $F_{(6, 106)}$=10.9, P<0.0001, (treatment X day) $F_{(6, 106)}$=0.69, P=0.65]. Results are mean ± SEM.



Supplementary Figure 17. Increasing adult hippocampal neurogenesis does not facilitate erasure of previously encoded memories. **a**, To test if increasing adult hippocampal neurogenesis facilitates erases or weakens previously encoded contextual fear memory, we performed two sets of experiments. In the first experiment, NCff mice were injected with Vehicle (n=12) or TAM (n=12) twenty-four hours following training (single foot shock in context A) and freezing behavior was measured in the training context at 22, 27 and 42 days after training. Both groups showed similar levels of

freezing at all time points tested [(Two-way repeated measures ANOVA, (treatment) $F_{(1, 22)=}0.7$, P=0.4, (day) $F_{(2, 44)=}28.4$, P<0.0001, (treatment X day) $F_{(2, 44)=}1$, P=0.34)]. **b**, In the second experiment, the strength of the fear memory was measured well within the window of hippocampal dependence of the fear memory and when the increase in adult hippocampal neurogenesis in *iBax*^{nestin} mice is maximal (8 weeks following TAM injection). A new cohort of NCff mice was injected with Veh (n=6) or TAM (n=8) six weeks prior to training and freezing behavior was measured at 7 and 21 days post training. Both groups showed similar levels of freezing behavior at 47 and 61 days post Veh/TAM injections [(Two-way repeated measures ANOVA, (treatment) $F_{(1, 12)=}0.17$, P=0.6, (day) $F_{(1, 12)=}1.45$, P=0.25, (treatment X day) $F_{(1, 12)=}0.3$, P=0.6)]. Results are mean ± SEM.

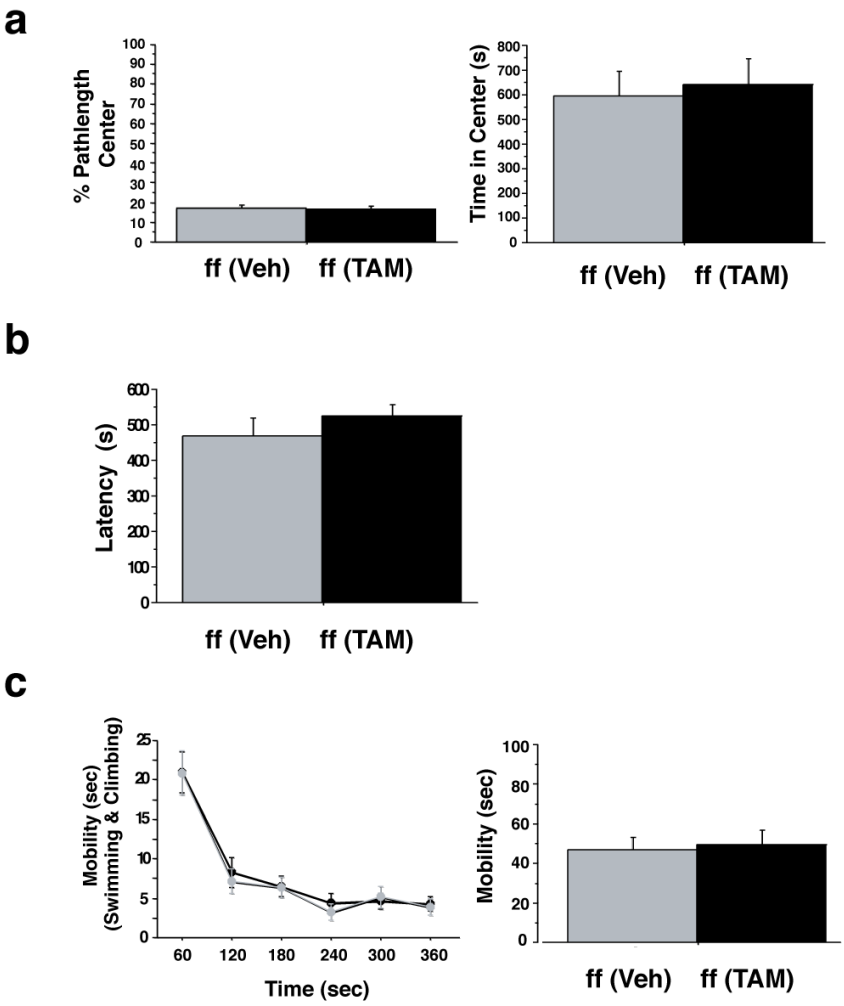


Supplementary Figure 18. *iBax*^{nestin} mice and controls spend similar amounts of time in the center of the Open Field and exhibit comparable anxiety-like behavior in the Light-Dark test and Elevated Plus Maze paradigms. **a**, Center time under low lighting conditions [(ANOVA $F_{(1, 24)=}0.039$, P=0.84), NCff+Veh:459 ± 58.2s, NCff+TAM:444.2 ± 47.1s)]. Center time under bright lighting conditions [(ANOVA $F_{(1, 17)=}1.2$, P=0.27), NCff+Veh:100.8 ± 35.3s, NCff+TAM:160.5 ± 38.5s)]. **b**, Light-Dark test: Time in light compartment [(ANOVA $F_{(1, 18)}<1$, P=0.84), NCff+Veh:50.3 ± 20.4s, NCff+TAM:57.2 ± 25.6s)]; % Pathlength in light compartment [(ANOVA $F_{(1, 18)}<1$, P=0.7), NCff+Veh:21.58 ± 4%,NCff+TAM:25.3 ± 7.6%)]; Total pathlength [(ANOVA $F_{(1,}$

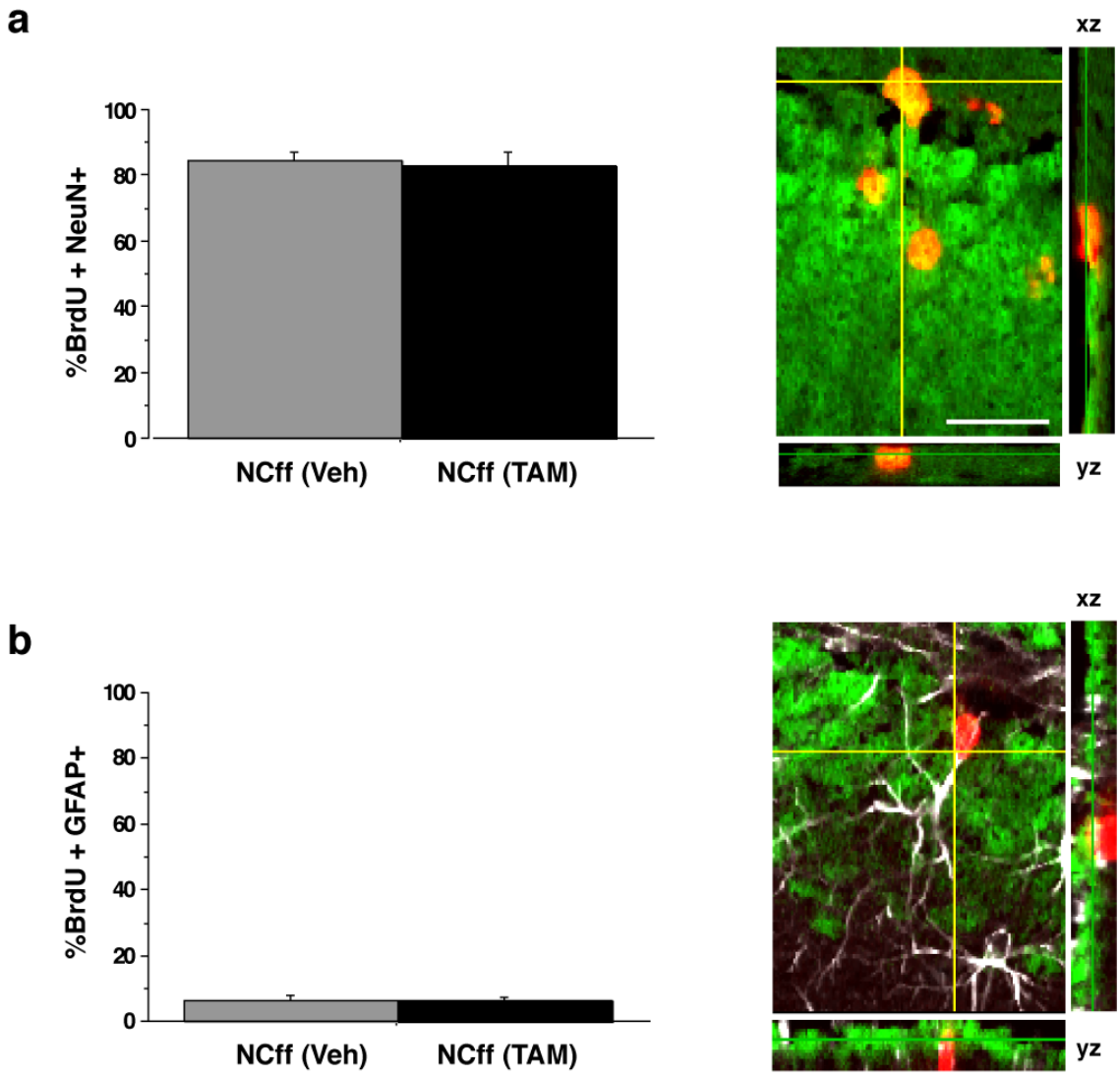
$_{18)}$<0, *P*=0.87), NCff+Veh:437.5 ± 28.1cm, NCff+TAM:448.4 ± 57.7cm)]; Entries in light compartment [(ANOVA $F_{(1, 18)}$=2.8, *P*=0.11), NCff+Veh:2.55 ± 0.338, NCff+TAM:4.27 ± 0.885)]. **c**, Elevated-Plus Maze: Time in open arms [(ANOVA $F_{(1, 18)}$<0, *P*=0.92), NCff+Veh:85.7 ± 24.8s, NCff+TAM:82.15 ± 24.2s)]; % Open arm entries [(ANOVA $F_{(1, 18)}$=1.2, *P*=0.28), NCff+Veh:61.1 ± 5%, NCff+TAM:67 ± 2.7%)]; Total arm entries [(ANOVA $F_{(1, 18)}$<1, *P*=0.46), NCff+Veh:14.66 ± 2.2, NCff+TAM:16.8 ± 1.9)]; Entries into open arms [(ANOVA $F_{(1, 18)}$<1, *P*=0.33), NCff+Veh:9.3 ± 1.5, NCff+TAM:11.2 ± 1.2)]. Results are mean ± SEM.

Statistical analysis for Figure 3.

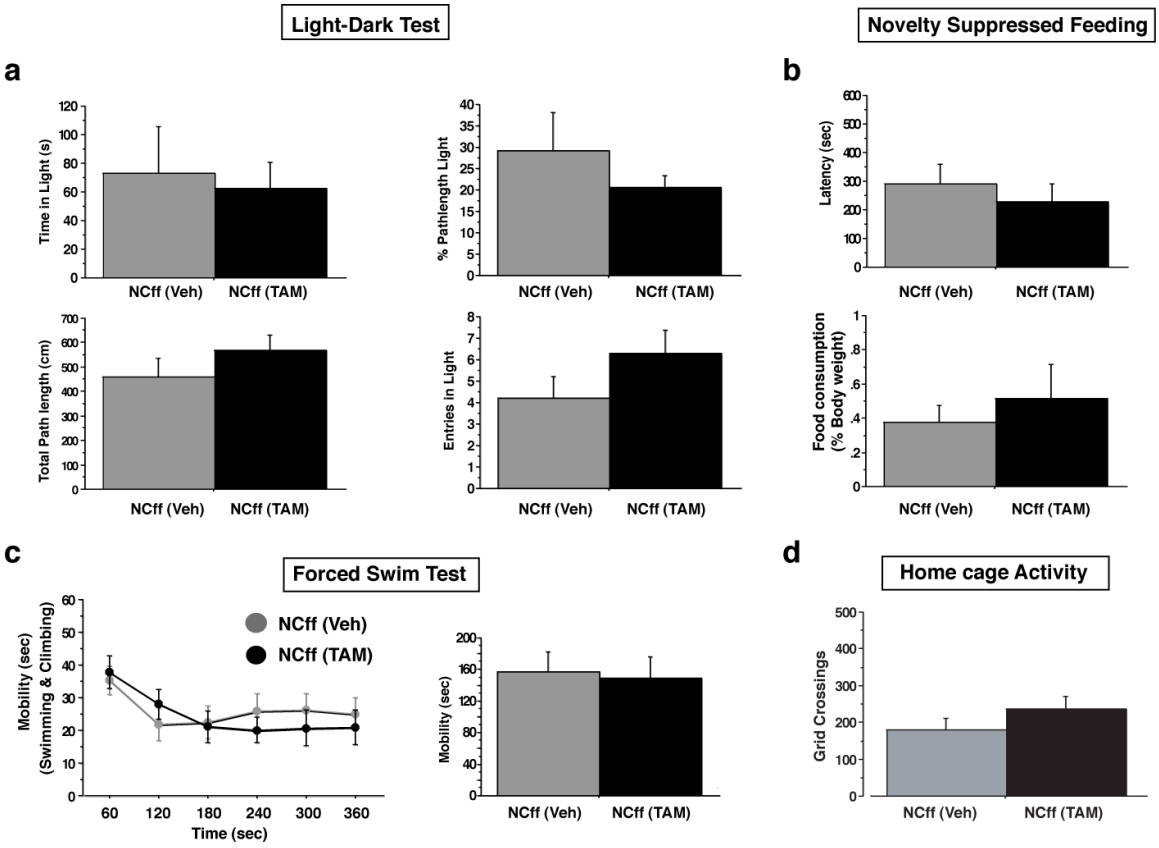
a, Open Field (low lux): Total path length [(repeated measures ANOVA $F_{(1, 24)}$=1.04, *P*=0.3, NCff+Veh:3769.9 ± 342.6cm, NCff+TAM:3290 ± 320cm)], Percentage path length center [(ANOVA $F_{(1, 24)}$<1, *P*=0.57, NCff+Veh:13.7 ± 1.2%, NCff+TAM:14.78 ± 1.4%)], Rearing events [(ANOVA $F_{(1, 24)}$<1, *P*=0.4, NCff+Veh:578.2 ± 63.2, NCff+TAM:511 ± 44)]. **b**, Open Field (high lux): Total path length [(repeated measures ANOVA $F_{(1, 17)}$<1, *P*=0.76, NCff+Veh:1162 ± 151.4cm, NCff+TAM:1240.2 ± 213cm)], Percentage path length center [(ANOVA $F_{(1, 17)}$=1.8, *P*=0.19, NCff+Veh:8.1 ± 2%, NCff+TAM:12 ± 1.9%)], Rearing events [(ANOVA $F_{(1, 17)}$<1, *P*=0.44, NCff+Veh:79.4 ± 20.6, NCff+TAM:104.8 ± 24.7)]. **c,** Novelty-Suppressed Feeding paradigm. Kaplan-Meier Survival analysis, Mantel-Cox log-rank test, *P*=0.41, Latency to eat [(ANOVA $F_{(1, 18)}$<1, *P*<0.05]. Home cage food consumption as % of body weight [(ANOVA) $F_{(1, 18)}$<1, *P*=0.57)]. **d**, Forced Swim test: (Mobility day 2) [(repeated measures ANOVA $F_{(1, 24)}$=3.4, *P*=0.07). Total mobility: NCff+Veh: 61.9 ± 5.57s, NCff+TAM: 46.8 ± 5.8s)]. n=9-14 mice per groups. Results are mean ± SEM.



Supplementary Figure 19. TAM treatment, on its own, does not affect anxiety-like and depression-like behavior. **a**, ff+Veh and ff+TAM mice showed similar anxiety-like behavior in the open field test. Main measures of anxiety-like behavior including "percentage path length center" and "time in center" were not significantly different between the two groups, [(ANOVA (percentage path length center) $F_{(1,26)}$=0.03, ANOVA (time in center) $F_{(1,26)}$=0.1)]. **b**, TAM treatment on its own does not affect latency to eat in the Novelty Suppressed Feeding paradigm, [(NCff+Veh:470.1 ± 49.76s, NCff+TAM:526.31 ± 31.3s, *P*=0.3, Unpaired 2-tailed Student's t-test)]. **c,** ff+Veh and ff+TAM mice showed comparable levels of mobility in the forced swim test on day 2 of testing. [(ANOVA $F_{(1,27)}$=0.066, *P*=0.79)], n=13-16 mice per group. Results are mean ± SEM.



Supplementary Figure 20. **a-b**, *iBax*^{nestin} mice and controls show a similar proportion of adult-born neurons and glial cells following a regimen of voluntary exercise. **a**, Percentage of adult-born neurons [(ANOVA $F_{(1, 8)}$<1, *P*=0.76), NCff+Veh:84.4.2 ± 2.3%, NCff+TAM:83 ± 3.7%)]. Representative confocal micrograph of BrdU (red)/NeuN (green) immunostained coronal hippocampal section of a TAM treated NCff mouse. **b**, Percentage of adult-born glial cells [(ANOVA $F_{(1, 8)}$<1, *P*=0.98), NCff+Veh:6.3 ± 1.2%, NCff+TAM:6.4 ± 1%)]. Representative confocal micrograph of BrdU (red)/GFAP (white) immunostained coronal hippocampal section of a TAM treated NCff mouse. n=5 mice per group. Results are mean ± SEM. Scale bars: 50μm**.**



Supplementary Figure 21. a-c, Following a regimen of voluntary exercise, *iBax*^{nestin} mice and controls exhibit similar anxiety-like behavior in the Light-Dark and Novelty suppressed Feeding paradigms and depression-like behavior in the Forced Swim test. **a**, Light-Dark test: Time in light compartment [(ANOVA $F_{(1, 19)}$<1, *P*=0.76), NCff+Veh:73.2 ± 32.1s, NCff+TAM:62.2 ± 18.4s)]; % Pathlength in light compartment [(ANOVA $F_{(1, 19)}$<1, *P*=0.34), NCff+Veh:29.2 ± 8.8%, NCff+TAM:20.5 ± 2.7%)]; Total pathlength [(ANOVA $F_{(1, 19)}$=1.2, *P*=0.28), NCff+Veh:460.3 ± 76.8cm, NCff+TAM:568.2 ± 61.8cm)]; Entries in light compartment [(ANOVA $F_{(1, 19)}$=1.8, *P*=0.18), NCff+Veh:4.2 ± 1, NCff+TAM:6.2 ± 1)]. **b**, Novelty suppressed Feeding paradigm: Latency to eat [(ANOVA $F_{(1, 19)}$<1, *P*=0.5), NCff+Veh:290.2 ± 69.7s, NCff+TAM:226.7 ± 64.2s)]; Food consumption in home cage as % of body weight [(ANOVA $F_{(1, 19)}$<1, *P*=0.55), NCff+Veh:0.377 ± 0.1%, NCff+TAM:0.5 ± 0.2%)]. Change in body weight [(ANOVA $F_{(1, 19)}$<1, *P*=0.45), NCff+Veh:5.29 ± 0.3g,

NCff+TAM:5.6 ± 0.25g)] **c**, Forced Swim test (Mobility day1, graph not shown) [(repeated measures ANOVA $F_{(1, 19)}<1$, $P=0.4$), Day 2 (above) [(repeated measures ANOVA $F_{(1, 19)}<1$, $P=0.8$)]. For a-c, n=10 (NCff+Veh) and n=11 (NCff+TAM). **d**, Home cage activity of NCff+Veh (n=7) and NCff+TAM (n=7) mice following voluntary exercise is comparable. Average number of grid crossings in home cage, ANOVA $F_{(1, 12)}=1.62$, $P=0.22$. Results are mean ± SEM.